DA CONT

increase neural crest stem cell numbers.

17. A method for isolating mammalian neural crest stem cells comprising:

(a) generating neural crest stem cells in accordance with the method of claim 1; and

(b) isolating the neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75.

18. A method for isolating rat neural crest stem cells comprising:

(a) generating rat neural crest stem cells in accordance with the method of claim 15; and

(b) isolating the rat neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75. --

REMARKS

Claims 1-6, 8-13 and 15 are pending in the instant application. Claims 1-6, 8-13 and 15 have been rejected. Claims 1, 9, and 15 have been amended. Claims 2 through 6 and 8 have been canceled, without prejudice. New claims 16 through 18 have been added. No new matter has been added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. Objection to Claims 2-5 under 37 C.F.R. § 1.75(c)

Claims 2-5 have been objected to under 37 C.F.R. § 1.75(c) as being of improper dependent form. Specifically, the Examiner suggests that the claims do not further limit the subject matter of the previous claim. Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have canceled claims 2 through 5 without prejudice. Withdrawal of this objection is therefore respectfully requested.

II. Rejection of Claims 1-6, 8-13 and 15 under 35 U.S.C. § 112, first paragraph

Claims 1-6, 8-13 and 15 have been rejected under 35 U.S.C. § 112, first paragraph, as the Examiner suggests that the specification does not enable any person skilled in the art to which it pertains, or with which its is most nearly connected, to make the invention commensurate in scope with the claims. The Examiner has acknowledged the specification to be enabling for the method of plating dissociated cells on a fibronectin substrate and a purification step on obtaining "pure, homogeneous populations of neuroepithelial cells", etc. However, the Examiner suggests that the specification does not reasonably provide enablement for a

method requiring "isolating a pure, homogeneous population of mammalian neural stem crest cells" by replating neuroepithelial cells onto laminin-coated or undefined substrates while removing FGF and/or chick embryo extract. The Examiner suggests that Kalyani et al. (1997), particularly page 219-220, teach that replating neuroepithelial cells onto laminin substrates, and/or removing FGF and/or chick embryo extraction does not result in a population of neural crest stem cells. The Examiner also suggests that Examples 5-7, Figure 1 and page 10 of the specification describe how withdrawal of CEE (as it relates to claim 1) or use of a "laminin-coated substrate" both lead to differentiation to motoneurons or glial, thus contradicting the recited steps for "generating said neural crest stem cells. The Examiner also refers to page 24, lines 7-8, of the specification as teaching that laminin promotes proliferation and neuronal differentiation and page 22, lines 14-16 as describing how use of fibronectin-substrate gave rise to a small percentage of GFAP-immunoreactive cells, which are not neural crest cells by definition.

Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended claims 1 and 15 to clarify that the steps used to generate neural crest stem cells include replating onto a fibronectin substrate and in a media comprising

chick embryo extract, NGF, FGF and EGF. In addition, Applicants have added new claims 16 through 18 drawn to a method for increasing neural crest stem cell numbers via addition of a dorsalizing agent and isolating neural crest stem cells via antibody capture with an antibody against neurotrophin receptor p75. Support for these amendments and new claims can be found in the specification at pages 50-63. Thus, no new matter has been added by these amendments.

The Examiner also suggests that the metes and bounds of dorsalizing agent is unknown as only three examples are listed in the specification. Applicants respectfully traverse this rejection.

At the outset, Applicants respectfully disagree with the Examiner's suggestion that only three examples of dorsalizing agents are described in the specification. The Examiner is respectfully directed to pages 61-62 of the specification wherein additional dorsalizing agents which promote crest differentiation are described. In addition, as evidenced by the following list of references, additional dorsalizing agents which promote crest differentiation such as wnt and retinoic acid were also known in the art at the time of filing this application. See e.g. Selleck et al. Proc. Natl Acad. Sci. USA 1996 93(18):9352-7; Chang et

al. Dev. Biol. February 1998 194(1):129-34; Saint-Jeannet et al. Proc. Natl Acad. Sci. USA 1997 94(25):13713-8; Maden et al. Curr. Biol. 1996 6(4):417-26; Rockwood and Maxwell Exp. Cell Res. 1996 223(2):250-8; and Deltour et al. FASEB J. 1996 10(9):1050-7. Copies of these references can be provided upon the Examiner's request.

Further, in an earnest effort to advance the prosecution of this case and in a effort to clarify the metes and bounds of dorsalizing agents as used in the instant application, claim 9 has been amended to state that dorsalizing agent is added to the cells to increase neural crest stem cell numbers. Similar language has been used in new claim 16 which depends from claim 15. Support for this language is found in the specification in Examples 23-24 at pages 57 through 63. Further, as taught at page 62, lines 9-11, the NEP cell-crest cell culture assay taught in the specification provides a means for one of skill in the art to identify additional dorsalizing agents with the claimed capabilities. Thus, the metes and bound of the term "dorsalizing agent" in the claims is now clear.

Withdrawal of these rejections under 35 U.S.C. § 112, first paragraph, is respectfully requested in light of the amendments to the claims and the above remarks.

III. Rejection of Claims 1-6, 8-13 and 15 under 35 U.S.C. § 112, second paragraph

Claims 1-6, 8-13 and 15 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite and incomplete for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner suggests that it does not appear that a "pure homogeneous population of mammalian/rat neuroepithelial stem cells" will be obtained from the recited steps because no purification step has been recited. Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended claims 1 and 15 to delete the phrase "isolated, pure homogeneous population" of mammalian/rat neuroepithelial stem cells as an isolated, pure homogeneous population was not required to generate neural crest stem cells as claimed. Support for this amendment can be found in Example 1 wherein the procedure for obtaining NEP cells is described.

The Examiner also suggests that claim 1(a)(iii) and claim 15(a)(iii) should read "feeder-cell-independent" culture for proper antecedent basis. Accordingly, in an earnest effort to advance the prosecution of this case, the claims have been amended to read "feeder-cell-independent culture".

With respect to claims 1-6 and 8-9, the Examiner suggests that the metes and bounds of "dorsalizing agent" is ambiguous in that the specification only lists three examples. Applicants respectfully disagree.

As discussed in Section II, *supra*, additional dorsalizing agents which promote crest differentiation are described at pages 61 and 62 of the specification. In addition, a list of references was provided in Section II, *supra*, evidencing the fact that the prior art has taught additional dorsalizing agents such as wnt and retinoic acid which promote crest differentiation.

Further, in an earnest effort to advance the prosecution of this case and in an effort to clarify the metes and bounds of dorsalizing agents as used in the instant application, claims relating to dorsalizing agents now state that the dorsalizing agent is added to the cells to increase neural crest stem cell numbers. Support for this language is found in the specification in Examples 23-24 at pages 57 through 63. In addition, as taught in the specification at page 62, lines 9-11, the NEP cell-crest cell culture assay taught in the specification provides a means for one of skill in the art to identify dorsalizing agents with the claimed capabilities.

MPEP § 2173 is clear; definiteness of claim language must be analyzed, not in a vacuum, but in light of:

(A) The content of the particular application disclosure;

(B) The teachings of the prior art; and

(C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

The metes and bounds of dorsalizing agents, as now set forth in the claims would be clear to one of skill in the art when read in light of the teachings of this application and those of the prior art.

The Examiner also suggests that claims 3-4 are indefinite because no antecedent basis for the recitation of "mitogen" exists in base claim 1. It is respectfully pointed out, however, that claims 3-4 have been canceled, thus mooting this rejection.

Withdrawal of these rejections under 35 U.S.C. § 112, second paragraph, is respectfully requested in light of the amendments to the claims and the above remarks.

IV. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly,

favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,

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Date: **February 25, 2002**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel claims 2 through 6 and 8.

Please amend the claims as follows:

1. (amended) A method for generating mammalian neural crest stem cells comprising:

(a) ~~isolating a pure, homogeneous population of~~ obtaining mammalian neuroepithelial stem cells derived from the neural tube from a mammalian embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a mammal at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the mammal; and

(iii) plating the dissociated cells in feeder-cell-independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract so that mammalian neuroepithelial stem cells are obtained; and

(b) inducing the ~~isolated, pure, homogeneous population of~~ neuroepithelial stem cells to differentiate in vitro by replating

the ~~isolated, pure, homogeneous population of~~ neuroepithelial stem cells ~~on laminin-coated substrate, withdrawing fibroblast growth factor or chick embryo extract from the isolated, pure, homogeneous population of neuroepithelial stem cells, or adding a dorsalizing agent to the isolated, pure, homogeneous population of neuroepithelial stem cells~~ onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF, thereby generating said neural crest stem cells.

9. (amended) The method of claim 1 wherein said inducing in step (b) further comprises adding a dorsalizing agent to the cells to increase neural crest stem cell numbers.

15. (amended) A method for generating rat neural crest stem cells comprising:

(a) ~~isolating a pure, homogeneous population of~~ obtaining rat neuroepithelial stem cells derived from the neural tube from a rat embryo at a stage of embryonic development after closure of the neural tube by:

(i) removing a sample of neural tube tissue from a rat at a stage of embryonic development after closure of the neural tube;

(ii) dissociating cells comprising the sample of neural tube tissue removed from the rat; and

(iii) plating the dissociated cells in feeder-cell-

independent culture on a substratum and in a media comprising fibroblast growth factor and chick embryo extract so that rat neuroepithelial stem cells are obtained; and

(b) inducing the ~~isolated, pure, homogeneous population of~~ neuroepithelial stem cells to differentiate in vitro by replating the ~~isolated, pure, homogeneous population of~~ neuroepithelial stem cells onto a fibronectin substrate and in a media comprising chick embryo extract, NGF, FGF and EGF, thereby generating said neural crest stem cells.